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(54) Title: LOW MOLECULAR WEIGHT POLYMANNURONATE

(57) Abstract: The present invention provides a process to prepare low molecular weight polymannuronate of average molecular weight between 10^3 and 10^5 through partial hydrolysis of alginate with organic acid, followed by the separation method using solubility difference dependent on pH. The low molecular polymannuronate has functions such as repressing obesity, lowering the levels of total cholesterol, triglyceride, phospholipid and LDL cholesterol, increasing HDL cholesterol, and decreasing GOT and GPT activities. Various kinds of functional foods and health-aids can be prepared using the low molecular polymannuronate.

LOW MOLECULAR WEIGHT POLYMANNURONATE

BACKGROUND

Field of the Invention

The present invention relates to low molecular weight polymannuronate, and more particularly, to a process for purifying low molecular weight polymannuronate from high molecular weight alginate through decomposition.

Description of the Relevant Art

The incidence of many cardiovascular diseases that are hard to treat such as hypertension, artheroscelrosis, angina pectoris, myocardial infarction and cerebral thrombosis, as well as obesity and diabetes, are increasing due to excessive nutrition of Western-style diet which includes high fat and high protein, and lack of exercise. There are increasing interests in the prevention and treatment of such diseases. It is preferable to prevent and/or treat these diseases with dietary foods supplemented with extracts from natural sources in lieu of chemically synthesized products, as such are safe methods without side-effects and decreased user reluctance to use such products.

Reflecting such trends, there have been much research and development focusing on dietary fiber. Dietary fiber was known to have preferable effects on the prevention of constipation and obesity as well as the prevention of geriatric diseases such as, thrombosis, artheroscherosis and hyperlipidemia (J. Ame. Clin. Nutr. 48:748-753, 1988; J. Ame. Clin. Nutr. 52:495-499, 1990; J. Ame. Clin. Nutr. 124:78-83, 1994).

Among dietary fibers, high molecular weight alginate, a dietary fiber

component constituting 20-30% cell-wall polysaccharides in marine algae (e.g. brown seaweed, sea tangle, gulfweed, hiziki), was known as having effects on lowering cholesterol levels *in vivo* and repressing obesity [J. Jap. Nutr. 26(3):78-83, 1974; J. Jap. Nutr. 33(6):273-281, 1974; Jap. J. Fisheries 59(5):879-884, 1993].

At present, many alginate-related products are being manufactured and sold. However, most of the alginates contained in the products are produced by simple extraction and processing from raw seaweed materials, and are high molecular weight alginates (more than about 400 million daltons). High molecular weight alginate is a block polymer consisting of mannuronate (M) and guluronate (G) monomers, and has high viscosity and low solubility in its high molecular weight form. Thus, it is not easy to add the high molecular form into foods (especially into beverages) in high concentrations.

Japanese Laid-Open Patent Publication Hei 6-7093 discloses a use of low molecularized alginate as supplement to functional drinks. The term "low molecularized alginate" as used herein refers to the state in which polyguluronate and polymannuronate of Mw. 10-900 kDa are mixed. Compared with high molecular weight alginate, low molecularized alginate is known to have lower viscosity and higher solubility, and to have increased functional effects on cholesterol levels.

Conventional methods used to prepare low molecularized alginate from high molecular forms include acid·alkali hydrolysis method [Haug, A., Larsen, B. and Smidsrod, O., Acta Chem. Scand., 20(1):183-190, 1966; Hirst, E. and Rees, D. A. J. Chem. Soc., 9:1182-1187, 1965; Hirst, E. L., Percival, E. and Wold, J. K. J. Chem.Soc., 8:1493-1499, 1964], hydrolysis under heat and pressure [Japanese Patent Laid-Open Publication Hei 6-7093, 1994; Kimura, Y., Watanabe, K. and Okuda, H., J. Ethnopharmacology 54:47-54,1996] and hydrolysis by enzymes [Doubet, R. S. and Quatrano, R. S., Appl. Environ. Microbiol. 47(4):699-703, 1984; Dunne, W. M. and Buckmire, F. L. A., Appl. Environ. Microbiol. 50(1):562-567, 1985; Hansen, J. B. and Nakamura, L. K. Appl. Environ. Microbiol. 49(4):1019-1021, 1985; Haug, A. and

Larsen, B., Carbohydr. Res. 17:297-308, 1971; Romeo, T. and Preston, J. F., Biochemistry 25(26):8385-8391, 1986; Yonemoto, Y., Murata, K., Kimura, A., Yamaguchi, H. and Okayama, K., J. of Fermen. and Bioengin. 72(3):152-157, 1991].

The acid·alkali hydrolysis method is hard to scale up to industrial scale due to deterioration of product quality, corrosion of reactors, need for neutralizing agents and troublesome handling for use of strong acids. The second method for preparing low molecularized alginate of Mw 10-900 kDa by heating at 100-200°C under pressure, also has defects, such as long reaction time and high-cost required for hydrolysis, as the process is carried out at high temperatures of more than 100°C under high pressure. The enzyme-hydrolysis method is not appropriate for industrialization due to long reaction time.

As mentioned above, compared with high molecular weight alginate, low molecularized alginate has increased effects on decreasing cholesterol levels and has improved physical properties such as solubility. Thus, the low molecularized alginate is expected to be useful for health-aid foods.

Low molecular weight polymannuronate, itself, has not been known as an essential element which has a direct effect on decreasing cholesterol levels, and a preparation method for extracting low molecular weight polymannuronate from natural alginate in highly pure quality has not been known before.

Polymannuronate is only known as a material for controlling the levels of toxic elements in patients with chronic uremia (Kulbe et al., U.S. Patent No. 4,689,322), or as a cell- or tissue-coating material to protect transplanted cells or tissue following transplantation (Dorian et al., U.S. Patent No.5,656,468).

However, in more advanced prospects, it is very desirable to identify and extract in pure form an essential component that has direct effect on decreasing cholesterol levels, from natural alginate. The present disclosure identifies the extraction of the

essential components in pure form so that they can be used directly as additives into foods. Thus, the present disclosure makes it possible to manufacture functional foods containing the essential component in more high-unit and in more accurate quantity. The resulting effect on related industry and health will be surprising. To this end, the present invention has the following objectives.

SUMMARY OF THE INVENTION

An object of the present invention is to provide a process for preparing low molecular weight polymannuronate in high purity.

Another object of the present invention is to use low molecular weight polymannuronate as a controller of serum cholesterol levels.

A further object of the present invention is to provide functional foods and health-aids containing such low molecular weight polymannuronate.

A comprehensive object of the present invention is to contribute to enhancement of a healthy life style and to prevent diseases by disclosing materials to be added into functional foods and health-aided foods that prevent and treat obesity, diabetes and cardiovascular diseases.

Further objects and advantages of the invention will become apparent to one skilled in the art after having the benefit of the present disclosure.

The present invention includes a process for preparing highly pure, low molecular weight polymannuronate, and a novel use thereof as controller of serum lipids. The present invention also includes functional foods and health-aids comprising this low molecular weight polymannuronate.

The process of the present invention relates to purification of only low molecular weight polymannuronate from high molecular weight alginate through decomposition using organic acids, followed by a separation using solubility differences dependent on pH. The process comprises the steps of adding an organic acid to high molecular weight alginate, heating the solution, adjusting the pH to between 2.5 and 3.5, and recovering the produced polymannuronate. The high molecular weight alginate is prepared from marine algae. Preferred organic acids include citric acid, malic acid, oxalic acid, lactic acid, succinic acid, tartaric acid and acetic acid. The preferred pH range is between 2.8 and 3.0

The present invention also includes compositions for preventing obesity, controlling serum lipids, enhancing liver function and expelling heavy metals from a body, comprising 0.01 to 100% by weight low molecular weight (M.W.) polymannuronate. The present invention also includes functional foods capable of controlling serum lipids and preventing hyperlipidemia, obesity and diabetes, comprising 0.01 to 100% by weight low M.W. polymannuronate. Such functional foods can be a dietary beverage or a solid food product. A health-aid for preventing and treating obesity, diabetes and cardiovascular diseases such as hypertension, arteriosclerosis, steniocardiac, myocardial infarction and cerebral thrombosis, comprising 0.01 to 100% by weight low M.W. polymannuronate, is also encompassed by the scope of this invention.

DETAILED DESCRIPTION OF THE INVENTION

According to the present process, high molecular weight alginate is partially hydrolyzed with an organic acid to produce low molecularized alginate, a mixture of polymannuronate and polyguluronate, from which only polymannuronate is separated by precipitation dependent on pH.

The high molecular weight alginate to be used as a starting material for the

present invention can be obtained from natural brown algae or from a dried-powder sample thereof after a proper preparation method which are well known in the art, i.e., extraction, neutralization, dehydration and drying under reduced pressure.

The organic acids which can be used for the present invention include, but are not limited to, citric acid, malic acid, lactic acid, oxalic acid, succinic acid, tartaric acid and acetic acid. Any organic acids that can hydrolyze high molecular weight alginate to low molecule weights can be used for the present invention. The degree of low-molecularization may be differentiated dependent on the organic acid chosen, but adjustable by controlling the concentration of the organic acid used or the hydrolysis time. As shown in one example of the present invention, acetic acid showed the maximum low-molecularization among various organic acids under the same concentration conditions. According to one embodiment of the present invention, suitable concentrations of the organic acid are between 0.2 mole and 2 mole, more preferably between 0.2 mole and 1 mole.

The hydrolysis of high molecular weight alginate with organic acid can be performed at a temperature between 80 to 120°C, and more preferably, between 95 to 105°C.

In the second step of the present process for preparing low-molecular weight polymannuronate, the pH of the resulting solution is adjusted between 2.5 and 3.5, more preferably between 2.8 and 3.0. If the pH is set under 2.5, the purity of the obtained polymannuronate will be high but the yield is low, and if the pH is over 3.5, the purity of polymannronate will be lowered. Since it is required to compromise the need for purity and yield of product, the indicated pH range was chosen.

The low-molecular weight polymannuronate obtained according to the process of the present invention is in high purity of over 90%. The "low-molecular" polymannuronate as used herein means a polymannuronate having average molecular weight between 1 and 100 kDa. The low molecular polymannuronate produced

according to the present invention is between 30 and 50 kDa, more preferably, 35 to 45 kDa.

The present inventors found that the low-molecular polymannuronate thus obtained is superior to materials such as high-molecular weight alginate, low molecularized alginate or low-molecular weight polyguluronate, in terms of the function to control serum lipid levels. The phrase "to control serum lipid levels" as used herein is intended to include many functions such as lowering the overall cholesterol level, increasing the levels of the advantageous high density lipoprotein (HDL), lowering the levels of low density lipoprotein (LDL) and controlling the levels of triglyceride and phospholipid, both in blood and liver, as well as lowering GOT and GPT values.

GOT and GPT values are results of measuring the activities of the enzymes, **G**lutamic **O**xalo**t**ransaminase and **G**lutamic **P**yruvic**t**ransminase, respectively. As the activities of GOT and GPT are increased in all types of injuries against liver and their increases are very sensitive to all damages, the ability to lower GOT and GPT values means an enhancement of liver function.

In animal experimentation, it was found that low-molecular polymannuronate is superior to low-molecularized alginate and low-molecular weight polyguluronate in terms of the function to control serum lipid levels. Further, it was also found that animal liver is not damaged with continuous and successive administration of low-molecular polymannuronate (see Example 3 below). The novel function of low-molecular polymannuronate according to the present invention is not only to lower the overall cholesterol level, but also to control advantageously the composition ratio of each type of cholesterol carrier. Further, the low-molecular polymannuronate can lower GOT and GPT values, and thus contribute to enhancement of liver function.

In addition, the present inventors found that the low-molecular polymannuronate according to the present invention associates with many harmful

heavy metals, such as Cd, Pb, Hg, and then eliminates them from the body. The affinity of polymannuranate to harmful heavy metals is much more increased compared with high-molecular weight alginate.

In addition, basically, as the binding ability of polymannuronate with water and water-keeping properties are high, polymannuronate is expected to be beneficial for relieving constipation.

As stated herein, the low-molecular polymannuronate obtained from the present invention is in high-purity and has functions related to controlling serum lipid levels. Also, it's physical properties such as water-solubility and viscosity is very good. Furthermore, it does not retain the peculiar smell and taste of natural brown algae. Thus, the low-molecular polymannuronate can be used as a functional additive to various foods or the low-polymannuronate powder alone, for the purpose of favorable adjust of serum lipid levels and prevention and/or treatment of obesity, diabetes.

The low molecular polymannuronate prepared according to the present invention may also be used as a material for controlling the levels of toxic elements in patients with chronic uraemia, or as a cell- or tissue-coating material to protect the transplanted cell or tissue from immune attack in transplantation.

The low-molecular polymannuronate prepared according to the present invention can be used as a main component or an additive or a supplement when various health-aid food and/or functional food are produced.

The term "functional food" as used herein, means a functionally-enforced special food in which the functionality of general food is enforced with the addition of polymannuronate. Functionality generally includes physical property and physiological functionality. The present polymannuronate has special viscosity and binding affinity to heavy metals as physical property, and has, as physiological functionality, a function to prevent hyperlipemia (i.e. resulting from lowering of

cholesterol levels), a function to enhance liver function, and so on. Therefore, if the polymannuronate of the present invention is added during a preparation of usual foods, the physical and physiological functionality of the usual food will be enhanced. As used herein, the term of "functional food" is defined to include all such functionally-enhanced foods not only in physical properties but also physiological functions. For example, the polymannuronate of the present invention may be added to the preparation of secondary-processed food, such as ham, in order to increase viscosity of the food and/or to prevent hyperlipemia/obesity. Then, this secondary processed food added with the present polymannuronate is generally referred to as functional food herein.

Separate from the definition for functional food, above, the term "health-aid foods" or "nutritionally special foods" mean health care foods which are made by adding polymannuronate to usual foods or by making an ingestable vehicle solely with polymannuronate. Such health-care foods are generally sought by patients or persons who have high likelihood of disease, to obtain a particular health effect. When treated with the present invention over an extended period of time, health-aid foods gives particular pharmacological effect just as medicine, but does not provoke a side-effect compared with usual medicine, because it is made of natural food.

For example, by using the function of the present polymannuronate to diet efficiency, we can produce functional food for diet. In addition, by using the function to enhance liver function, we may produce functionality-enforced foods or beverage.

Also, the polymannuronate according to the invention is applicable for the preparation of a health-aid food that can be used as one of diet therapy to lower and/or adjust cholesterol levels in patients of hyperlipidemia or to prevent hyperlipidemia.

Examples of other applications include dietary fiber beverage for prevention of constipation, cholesterol-lowering functional bread, flour noodles and magarine.

The low-molecular polymannuronate according to the invention is preferably

contained in the final functional foods or health-aided foods in a proportion between 0.01-100% of final foods. More preferred proportion is generally dependent on the group of foods, i.e. more or less of 0.01-5% for drinks, more or less of 10-50% for noodles, more or less of 40-100% for health-aid foods.

Functional flour-derived foods such as noodles and bread containing low-molecular polymannuronate can be prepared through mixing the present polymannuronate in powder form and conventional flour in proportions of 1 to 50% of low-molecular polymannuronate, and then preparing final noodles and bread dough from the mixed powder using conventional methods. As used herein, the term "solid food product" encompasses such functional foods as noodles and bread, but are not limited thereto. One skilled in the art will be able to easily substitute the given examples with other food substances, after having the benefit of this disclosure.

A capsule- or a tablet-formed diet food comprising low-molecular polymannuronate according to the invention as a main ingredient and optionally common additives can be prepared by using conventional manufacturing method known in the art.

Including the above applications, the present low-molecular poly-mannuronate can be applied in diverse field of food industry in its original powder form or a dissolved solution dependent on needs. For example, it can be added into usual beverages to produce functional beverages, can be added into high fat/cholesterol foods, such as ham and sausage to reduce cholesterol level, and also can be added into seasonings for meat or a salt.

The present invention will be described in greater detail by way of the following examples, which are not intended to limit the invention.

Example 1

1. Preparation of low-molecular polymannuronate

About 60g of alginate (molecular weight of about 1300 kDa) was mixed with 600 ml of each organic acid solution in each concentration as shown in table 1 to table 6 below. The mixture was stirred and was hydrolyzed at a temperature of about 100°C for the times indicated in each column of tables (as the concentration of organic acid is inversely proportional to the hydrolysis time, the thicker is the concentration of organic acid, the shorter the hydrolysis time). The resulting low-molecularized alginate solution in the state of a mixture of low-molecular polymannuronate and low-molecular polyguluronate was adjusted to pH 2.8-3.0 with addition of same organic acid and then centrifuged for separation (the upper liquid section is polymannuronate and the lower precipitation section is polyguluronate). The supernatant was collected, neutralized by addition of sodium carbonate (1 M), added with ethanol up to final concentration of 50% to produce precipitation, and centrifuged to obtain the precipitation.

The obtained precipitation was dissolved in a minimum amount of distilled water (about 200 ml). The resulting solution was adjusted to pH 2.8-3.0 with the same organic acid and centrifuged for separation. The supernatant was neutralized by the addition of sodium carbonate (1M), added with the same volume of ethanol as above to give precipitation, which is separated by centrifugation, to obtain low-molecular polymannuronate.

2. Molecular weight measurement of obtained polymannuronate

The molecular weight of obtained polymannuronate was measured by using Sepharose CL-4B and Sepharose CL-6B column chromatography (φ 12 mm × 97.6 cm) and Pullulan (Shodex standard P-82) as standard. The average molecular weight of polymannuronate prepared according to the present invention was 46.1 kDa.

3. Purity assay of obtained polymannuronate

After dissolving the obtained low-molecular weight polymannuronate in 1% triethylamine solution, the purity and composition of the obtained low-molecular polymannuronate was analyzed by HPLC using Whatman Partisil 10-SAX anion exchange column (250 × 4.6 mm i.d.) and using 0.02 mole potassium phosphate buffer (pH 4.6) containing 5% methanol. By standard, chromatogram of guluronate lactone and mannuronate lactone (Sigma Co.) analyzed by the same HPLC as that of the sample analysis was compared with each elution pattern of each sample, to determine purity. The average purity of polymannuronate produced according to the present invention was 91% in 1 hr, 93% in 3 hrs and 96% in 5 hrs depending on hydrolysis time.

Example 2

Partial hydrolysis of high molecular weight alginate with various organic acids

Using diverse kinds of organic acids partial hydrolysis of high-molecular weight alginate was performed for identical hydrolysis time, the result of which is shown in table 1 below. Dependent on the used organic acid, the progress of low-molecularization was differed, obtaining the maximum low-molecularization with the use of acetic acid in the same concentration. Their yields were similar near 80%.

[Table 1]

Relationship between organic acids and molecular weight of polymannuronate

Organic acid (0.4 M)	Hydrolysis time (hrs.)	Molecular weight (kDa)
Citric acid	3	24.0
Malic acid	3	53.2
Oxalic acid	3	37.6
Lactic acid	3	33.8
Succinic acid	3	35.4
Tartaric acid	3	33.1

Acetic acid	3	7.5

* Reaction was performed under constant temp. of 100°C.

The hydrolysis was performed with diverse concentration of acetic acids (0.2~1.0 M), the results of which is shown in table 2 below. As can be seen from table 2, the degree of low-molecularization of alginate was increased with the increase in the concentration of organic acid. In case of acetic acid, as low concentration as 0.2 M is sufficient to produce the low molecular polymannuronate of 40 kDa.

[Table 2]

Relationship between acetic acid concentration and produced polymannuronate

Acetic acid conc.(M)	Hydrolysis time (hrs)	Molecular weight (kDa)
0	0	1,283.0
0.2	3	40.0
0.4	3	7.5
0.6	3	3.8
0.8	3	1.9
1.0	3	0.6

* Reaction was performed under constant temp. of 100°C.

Varying hydrolysis time, the partial hydrolysis of high molecular weight alginate was performed with unvaried concentration of acetic acid, malic acid, oxalic acid and citric acid, the results of which are shown in Tables 3 to 6, respectively. The degree of low-molecularization increased with extension of reaction time from 10 to 240 minutes. Especially, more rapid hydrolysis (low-molecularization) was achieved at the beginning of reaction (10~60 min).

[Table 3]

Relationship between hydrolysis time and molecular weight of produced polymannuronate (using acetic acid)

Acetic acid conc.(M)	Hydrolysis time (min)	Molecular Weight (kDa)
0.4	0	1,283.90
	10	462.1
	20	185.6
	40	109.0
	55	43.2
	60	32.8
	120	23.7
	180	7.5
	240	4.4

* Reaction was performed under constant temp. of 100°C.

[Table 4]

Relationship between hydrolysis time and molecular weight of produced polymannuronate (using malic acid)

Malic acid conc.(M)	Hydrolysis time (min)	Molecular weight (kDa)
0.4	0	1,283.0
	20	569.0
	40	446.1
	60	234.2
	120	123.9
	180	53.2
	240	24.0

* Reaction was performed under constant temp. of 100°C.

[Table 5]

Relationship between hydrolysis time and molecular weight of produced polymannuronate (using oxalic acid)

Oxalic acid conc.(M)	Hydrolysis time (min)	Molecular weight (kDa)
0.4	0	1,283.0
	20	465.4
	40	354.8
	60	162.8
	120	82.5
	180	37.6
	240	15.4

* Reaction was performed under constant temp. of 100°C.

[Table 6]

Relationship between hydrolysis time and molecular weight of produced polymannuronate (using citric acid)

Citric acid's conc.(M)	Hydrolysis time(min)	Molecular weight (kDa)
0.4	0	1,283.0
	20	452.4
	40	332.8
	60	154.0
	120	78.5
	180	24.0
	240	13.2

* Reaction was performed under constant temp. of 100°C.

Example 3

The effect of low-molecular polymannuronate (animal test)

1. Materials and Methods

(1) Composition of experimental diet

Compositions and Contents of a basal diet, a cholesterol diet and a experimental diet are shown in table 7 below. The cholesterol diet (control) was prepared by adding 1% cholesterol to basal diet and substracting the same amount of sucrose from the basal diet. Experimental diet was prepared by adding 1% cholesterol and one selected from a group consisting of 5% low molecular polymannuronate (pM), 5% polyguluronate (pG) and mixture of 2.5% pM and 2.5% pG to basal diet, and by substracting the corresponding amount of sucrose from basal diet.

[Table 7]

Composition of experimental diet (g/kg)

Dietary component	Testing animal group				
	basal diet	Control	PM diet	pM+pG diet	pG diet
Casein	180	180	180	180	280
Rad oil	80	80	80	80	80
Corn oil	20	20	20	20	20
Mineral	40	40	40	40	40
Vitamin	8.5	8.5	8.5	8.5	8.5
Choline chloride	2	2	2	2	2
Cholesterol	0	10	10	10	10
Sodium cholinate	0	2.5	2.5	2.5	2.5
Polymannuronate	0	0	50	25	0
Polyguluronate	0	0	0	25	50
Sugar	669.5	657	607	607	607

(2) Experimental animal

Male Sprague Dawley (SD) rats aged 4 weeks (purchasing from Korean Experimental Animal Institute) were used as test animals in the present

experimentation. A total of 50 animals were divided into five groups as indicated in the table 7 above, and each group of animals were fed with the diet for five (5) weeks, the composition of which is shown in the above table 7.

The living condition of test animal was 22±2°C of room temperature and 65±3% of humidity, which were autoregulated. After five weeks of diet, bloods from test animals were collected, from which serums were separated, and an examination of the levels of cholesterol, of triglyceride, of phospholipid and of low density lipoprotein were performed both in serum and liver sample.

For the test of the levels of cholesterol, of triglyceride, of phospholipid and of low density lipoprotein, kit reagent (produced by Shin-yang Chemical, Inc.) was used, and the food grade diets were used to feed test animals.

(3) Total cholesterol level and free cholesterol levels:

For examination of total cholesterol and free cholesterol levels in serum and liver extraction sample, 100 $\mu\ell$ of serum and liver extraction sample were used for examination by cholesterol CII-test kit and by free cholesterol C-test kit (produced by Shin-yang Chemical, Inc.).

(4) Triglyceride and phospholipid levels:

For examination of triglyceride and phospholipid levels, 100 $\mu\ell$ of serum and liver extraction sample were used for examination by triglyceride G-test kit and by phospholipid C-test kit (produced by Shin-yang Chemical, Inc.).

(5) High-density lipoprotein and low-density lipoprotein cholesterol levels:

The level of high-density lipoprotein cholesterol was examined by using high-density lipoprotein cholesterol C-test kit (produced by Shin-yang Chemical, Inc., Korea) for 100 $\mu\ell$ of serum and liver extraction sample. The level of low-density

lipoprotein cholesterol was calculated by substracting the level of high-density lipoprotein from the level of total cholesterol.

(6) Activities of Glutamic oxaloacetic transaminase (GOT) and Glutamic pyruvic transaminase (GPT):

The activities were measured by using the GOT and GPT activity test kits for each 100 $\mu\ell$ of serum sample taken.

(7) Statistical evaluation:

The data of experiments were statistically processed by calculation of mean and standard deviation for each test group. Statistical significance of each test group was evaluated by Duncan's multiple test ($p<0.01$).

1. The obesity-suppressing effect of low-molecular polymannuronate

Weight increases in five test groups of animals were inspected, the results of which is shown in table 8. As can be seen from the table, the test group animals dieted with 5% low-molecular polymannuronte for five weeks resulted in efficient suppression of weight increase, compared with the control group.

[Table 8]

Feed efficiency during the period of 5-weeks of feeding

Testing group	Body Weight Increase	Feed intake	Feed efficiency
Basal diet	197.2	429.1	0.46
Control[*1]	212.6	433.8	0.49
Polymannuronate[*2]	199.0	446.8	0.44

Polymannuronate+ Polyguluronate*3	201.6	453.4	0.44
Polyguluronate*4	200.1	442.1	0.45

*1 Test group dieted with basal diet + Cholesterol 1%.

*2 Test group dieted with basal diet + Cholesterol 1% + Polymannuronate 5%.

*3 Test group dieted with basal diet + Cholesterol 1% + Polymannuronate 2.5% + Polyguluronate 2.5%.

*4 Test group diested with basal diet + Polyguluronate 5%.

2. Effects on cholesterol level

In five test groups of animals were inspected, the results of which is shown in table 9. As can be seen from the table, the test group animals dieted either with 5% low-molecular polymannuronte or with 5% polyguluronate or with both for five weeks resulted in efficient lowering of cholesterol levels compared with the control group. Especially, the degree of lowering cholesterol level was the highest in the group dieted the low-molecular polymannuronate according to the present invention. Also, the lowering effect in the group fed with admixed diet of polymannuronate + polyguluronate was higher than that in the group fed only with polyguluronate. From these results, it is expected that the essential component in the low molecularized alginate (i.e. admixture of polymannuronate and polyguluronate) that lowers cholesterol levels in the serum and liver is the low-molecular polymannuronate component of the present invention. According to the results of the present test, the diet of low-molecular polymannuronate of the present invention lowered the serum cholesterol level by 46%, and liver cholesterol level by 59% compared with the levels in control group.

[Table 9]

Cholesterol levels in the serum and liver of the rats fed the experimental diet

Testing group	serum (mg/dl)	liver (mg/g)
Basal diet	35.1 ± 1.3	7.4 ± 0.2
Control*1	284.2 ± 3.6	35.6 ± 0.3
Polymannuronate*2	153.3 ± 2.7	14.7 ± 0.2
Polymannuronate + polyguluronate *3	207.5 ± 3.3	19.6 ± 0.2
Polyguluronate *4	218.8 ± 3.4	22.1 ± 0.3

*1, *2, *3 and *4. Refer to the footnote of Table 8

3. Effects on triglyceride and phospholipid

The levels of triglyceride and phospholipid in the serum and liver in the five test groups of animals were inspected, the results of which are shown in tables 10 and 11, respectively.

As can be note from table 10, the level of triglyceride in the serum was the highest in cholesterol diet group and the lowest in polymannuronate diet group. The levels of triglyceride in the other two testing feed groups (i.e. admixture feeding and polyG feeding groups) were shown to be similar to that of basal diet group. Similarly, the level in liver extract was the highest in cholesterol diet group and the lowest in polymannuronate diet group.

As can be noted from table 11, the levels of phospholipid in both serum and liver were the highest in cholesterol diet group and the lowest in basal diet group. The levels of phospholipid in all three testing feed groups were lower than that of cholesterol diet group, and particularly, the lowest in polymannuronate diet group.

With the administration of the low-molecular polymannuronate of the present invention, the levels of triglyceride and phospholipid in the serum were decreased by 42% and 48%, respectively, and the levels in liver were decreased by 35% and 40%, respectively, compared with those of control group.

[Table 10]

Triglyceride levels in the serum and liver of rats fed with experimental diet (Means ± S.E.)

Testing group	serum (mg/dl)	liver (mg/g)
Basal diet	62.5 ± 3.4	42.3 ± 1.3
Control*1	93.3 ± 4.2	79.2 ± 2.0
Polymannuronate*2	54.3 ± 2.4	40.8 ± 1.7
Polymannuronate + polyguluronate*3	60.0 ± 2.7	49.2 ± 1.9
Polyguluronate*4	72.1 ± 2.9	51.9 ± 1.9

*1, *2, *3 and *4. Refer to the footnote of Table 8

[Table 11]

Phospholipid levels in the serum and liver of rats fed with experimental diet (Means ± S.E.)

Testing group	serum (mg/dl)	Liver (mg/g)
Basal diet	48.9 ± 1.5	10.2 ± 0.8
Control*1	98.8 ± 3.2	24.5 ± 1.5
Polymannuronate*2	63.8 ± 2.6	14.8 ± 0.9
Polymannuronate + polyguluronate*3	68.5 ± 2.9	15.7 ± 0.7
Polyguluronate*4	68.0 ± 3.0	18.6 ± 0.7

*1, *2, *3 and *4. Refer to the footnote of Table 8

4. Effects on the levels of high-density lipoprotein and low-density lipoprotein cholesterol.

The levels of high-density and low-density lipoprotein cholesterol both in serum and in liver were investigated in the five groups of animals, the results of which

are shown in table 12 and 13.

The level of high-density lipoprotein in serum was the lowest in the cholesterol diet group and the highest in the polymannuronate diet group, whereas the level in liver was the lowest in basal diet group and the highest in the polymannuronate diet group (see table 12).

The levels of low-density lipoprotein both in serum and in liver were the highest in the cholesterol diet group and the lowest in the basal diet group (see table 13). Compared with the cholesterol diet group (control), in the three testing group feeding with polyM and/or polyG diet, the low-density lipoprotein levels were significantly decreased, with the most outstanding decrease in low-molecular polymannuronate diet group.

Compared with the control (cholesterol diet), with the diet of low-molecular polymannuronate according to the invention, the serum levels of high-density lipoprotein cholesterol were increased by 4.5 times and serum levels of low-density lipoprotein cholesterol decreased by 59%. Further, the high-density lipoprotein cholesterol level in liver was increased by 1.2 times, and that of low-density lipoprotein decreased by 47%.

[Table 12]

HDL-cholesterol levels in the serum and liver of rats fed the experimental diet (mean ± S.E.)

Testing group	HDL cholesterol in serum (mg/dl)	HDL cholesterol in liver (mg/g)
Basal diet	27.8 ± 1.1	3.3 ± 0.1
Control*1	8.6 ± 0.2	5.7 ± 0.3
Polymannuronate*2	39.4 ± 0.9	6.8 ± 0.2

Polymannuronate + polyguluronate*3	23.5 ± 0.6	5.4 ± 0.4
Polyguluronate*4	15.2 ± 0.7	4.9 ± 0.3

*1, *2, *3 and *4. Refer to the footnote of Table 8

[Table 13]

LDL-cholesterol levels in the serum and liver of rats fed the experimental diet (mean ± S.E.)

Testing group	LDL cholesterol in serum (mg/dl)	LDL cholesterol in liver (mg/g)
Basal diet	7.3 ± 0.3	4.1 ± 0.3
Control*1	275.6 ± 3.4	29.9 ± 0.5
Polymannuronate*2	113.9 ± 1.4	7.9 ± 0.2
Polymannuronate + polyguluronate*3	184.0 ± 2.4	14.2 ± 0.3
Polyguluronate*4	203.6 ± 2.9	17.2 ± 0.3

*1, *2, *3 and *4. Refer to the footnote of Table 8

5. Effects of low-molecular polymannuronate on serum GOT and GPT values

The activities of GOT and GPT in the serum of five testing groups were investigated, the results of which are shown in table 14. As shown in table 14, the effect on lowering GOT and GPT activities is the highest in the low-molecular polymannuronate diet group, i.e. 38% decrease in GOT and 30% decrease in GPT compared with control.

[Table 14]

Activities of GOT and GPT in the serum of the rats fed with the experimental diet (Mean ± S.E.)

Testing group	GOT (Karmen)	GPT (Karmen)
Basal diet	23.6 ± 1.7	18.5 ± 1.4
Control*1	45.2 ± 2.3	23.4 ± 2.5
Polymannuronate*2	27.8 ± 2.1	16.3 ± 1.5
Polymannuronate + polyguluronate*3	31.9 ± 1.8	18.5 ± 1.8
Polyguluronate*4	33.4 ± 2.0	18.8 ± 1.9

*1, *2, *3 and *4. Refer to the footnote of Table 8

6. **Acute toxicity test**

To each of 4-week old ICR mouse (80 males), a single dose of 2g/kg of low-molecular polymannuronate of the present invention was orally administered. After administration, each mouse was carefully observed at each hour during first 6 hours and for 2 weeks, with respect to its general condition, motility, body weight, appearance and symptoms in auto nervous systems. From daily observation for 2 weeks after oral administration, no abnormalities in motility, body weight, tremor and reflex reaction were observed. As a result, LD50 was more than 2000mg/kg from this acute toxicity test.

Example 4.

Test on the heavy metal affinity of polymannuronate

The purified seaweed alginate, polymannuronate and polyguluronate were dis solved in distilled water and their concentration were adjusted to 400 μg /ml. Metal salts were dissolved in distilled water to prepare for the solutions with con centrations of 0 to 50 or 100 mM. Cations used in this study were Ca^{2+}, Cd^{2+}, Co^{2+}, C u^{2+}, Fe^{3+}, Hg^{2+}, Mg^{2+}, Mn^{2+}, Pb^{2+}, Rb^{1+}, Sr^{2+} and Zn^{2+}. Four volumes of seaweed alginat e, polymannuronate and polyguluronate solution were mixed with one volume of each

cation solution, respectively. The mixture was incubated 2 hr at room temperature an d centrifuged (1,800 x g for 20 min). The concentration of polymer in each supernata nt was measured by phenol-sulfuric acid method [Dubois et al., *Anal. Chem.*, 28, 350-356, 1956]. The concentration of precipitated polymer was calculated and its value w as used for determination of relative affinity of polymers for cations. Table 15 shows the result.

[Table 15]

The precipitation of polymer by heavy metal ion

Heavy metal ion	Concentration(nM)*		
	Polymannuranate	Polyguluronate	Alginate
Ca	8.0	8.5	17.6
Cd	3.5	3.6	3.6
Co	20.2	9.9	11.5
Cu	3.5	4.6	3.2
Fe	2.7	3.4	2.7
Hg	18.0	77.7	100<
Mg	100<	100<	100<
Mn	37.2	90.2	63.5
Ru	15.5	16.9	24.1
St	15.6	16.6	23.1
Zn	15.2	18.3	14.5
Pb	5.2	5.5	5.3

* the concentration of metal ion required to precipitate 50% of polymer from each 400 μg/ml solution of polymer (polymannuronate, polyguluronate and alginic acid)

With reference to the above table 15, the affinities of polymannuronate to Fe, Cu, Cd, Pb and Ca are outstanding, and its affinities to Zn, St, Ru, Hg, and Co are relatively good, while affinity to Mg is weak. Polyguluronate showed similar tendency to polymannuronate except its lower affinity for Mn and Hg. The affinity of alginic acid to heavy metal ions was much lower than that of polymannuroate and

polyguluronate.

As fully illustrated hereinabove, the present invention has specific advantages. The process for preparing low-molecular polymannuronate according to the invention has advantages over the prior methods using inorganic acid such as HCl or sulfonic acid, in that problems such as corrosion of machinery including reactor due to strong inorganic acids and post-process treatment for neutralization are expelled. The present process is also advantageous over the prior methods using enzyme or high pressure/temperature in terms of hydrolysis time and cost.

According to the present process, a highly pure (90% or more) polymannuronate, which is gradually low-molecularized into desired degree, can be prepared.

The low-molecular polymannuronate prepared according to the present process is a single material of high purity with high solubility, has increased functional effects such as an effect on lowering cholesterol, as an effective ingredient of natural alginate, and does not retain peculiar flavor and taste of natural alginate. Thus, when it is used as additives for the preparation of functional foods and health-aided foods, the functionality of foods can be controlled more accurately and desired functional effect could be obtained with a use of less amount. Accordingly, it is the best of choice material for the preparation of functional foods and health-aided foods.

* * * * * *

The present invention has been described with reference to various specific examples. However, it should be understood that numerous variations and modifications are possible to those skilled in the art without departing from the spirit of the present invention, and all such variations and modifications are intended to be within the scope of the claims which follow.

What is claimed is:

1. A process for preparing low molecular polymannuronate from high molecular weight alginate, comprising the steps of:

(1) adding an organic acid to high molecular weight alginate and heating;

(2) adjusting the pH to between 2.5 and 3.5; and

(3) recovering the produced polymannuronate.

2. The process according to claim 1, wherein the high molecular weight alginate is prepared from marine algae.

3. The process according to claim 1, wherein the organic acid is selected from the group consisting of citric acid, malic acid, oxalic acid, lactic acid, succinic acid, tartaric acid and acetic acid.

4. The process according to claim 1, wherein the pH is between 2.8 and 3.0

5. A composition for preventing obesity, controlling serum lipids, enhancing liver function and expelling heavy metals from a body, comprising 0.01 –100% by weight low M.W. polymannuronate.

6. A functional food capable of controlling serum lipids and preventing hyperlipidemia, obesity and diabetes, comprising 0.01 –100% by weight low M.W. polymannuronate.

7. The functional food according to claim 6 which is a dietary beverage.

8. The functional food according to claim 6 which is a solid food product.

9. A health-aid for preventing and treating obesity, diabetes and cardiovascular diseases such as hypertension, arteriosclerosis, steniocardiac, myocardial infarction and

cerebral thrombosis, comprising 0.01 –100% by weight of low M.W. polymannuronate.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/KR01/00139

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 A23L 1/29, C08B 37/04, C07H 1/08, A61K 35/80, A23L 1/325

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimun documentation searched (classification system followed by classification symbols)

IPC7 A23L 1/29, C08B 37/04, C07H 1/08, A61K 35/80, A23L 1/325

Documentation searched other than minimun documentation to the extent that such documents are included in the fileds searched

Electronic data base consulted during the intertnational search (name of data base and, where practicable, search trerms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 96/37519 A (FIDIA ADVANCED BIOPOLYMERS S.R.L.) 28 November 1996 see the whole document	1-9
A	US 3948881 A (UNIROYAL, LTD.) 6 April 1976 see the whole document	1-4
A	JP 63-233797 A (NICHIDEN KAGAKU) 29 September 1988 see claim 2	1-4
A	JP 6-172375 A (KIBUN FOODS INC.) 21 June 1994 see the whole document	1-4
A	JP 55-131360 A (KIBUN FOODS INC.) 13 October 1980 see claims 3-5	5-9

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
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INTERNATIONAL SEARCH REPORT

Information on patent family members

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